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Thermal preference of *Culicoides* biting midges in laboratory and semi-field settings

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A B S T R A C T

Biting midges of the genus *Culicoides* (Diptera: Ceratopogonidae) are hematophagous insects, and some species can transmit a plethora of pathogens, e.g., bluetongue virus and African horse sickness virus, that mainly affect animals. The transmission of vector-borne pathogens is strongly temperature dependent, and recent studies pointed to the importance of including microclimatic data when modelling disease spread. However, little is known about the preferred temperature of biting midges. The present study addressed the thermal selection of field-caught *Culicoides* with two experiments. In a laboratory setup, sugar-fed or blood-fed midges were video tracked for 15 min while moving inside a 60 × 30 × 4 cm setup with a 15–25 °C temperature gradient. *Culicoides* spent over double the time in the coldest zone of the setup compared to the warmest one. This cold selection was significantly stronger for sugar-fed individuals. Calculated preferred temperatures were 18.3 °C and 18.9 °C for sugar-fed and blood-fed *Culicoides*, respectively. The effect of temperature on walking speed was significant but weak, indicating that their skewed distribution results from preference and not cold trapping. A second experiment consisted of a two-way-choice-setup, performed in a 90 × 45 × 45 cm net cage, placed outdoors in a sheltered environment. Two UV LED CDC traps were placed inside the setup, and a mean temperature difference of 2.2 °C was created between the two traps. Hundred-fifty *Culicoides* were released per experiment. Recapture rates were negatively correlated with ambient temperature and were on average three times higher in the cooled trap. The higher prevalence of biting midges in cooler environments influences fitness and ability to transmit pathogens and should be considered in models that predict *Culicoides* disease transmission.

1. Introduction

The hematophagous biting midges of the genus *Culicoides* (Diptera: Ceratopogonidae) are worldwide distributed vectors of mainly animal pathogens, such as bluetongue virus (BTV), African horse sickness virus, epizootic haemorrhagic disease (EHD) virus (Mellor et al., 2000) and Schmallenberg virus (SBV) (Endalew et al., 2019). Since 1998, European livestock has experienced several outbreaks of BTV (Ganter, 2014), for which multiple species of the *Culicoides* genus have been identified as competent vectors (Mellor, 1990; Paslaru et al., 2018). One major wave of BTV started in the Netherlands in 2006 and swept across numerous European countries, leading to the death of 1.5 million sheep (Ganter, 2014). In response, countries have adopted surveillance programs, restrictions to livestock movement, and vaccinations, aiming to reduce the spread of the virus (Sick et al., 2019). A new outbreak has been declared as recently as October 2023, in the Netherlands (Stokstad, 2023). Despite these emedrging issues, the knowledge about biting midges' ecology and behaviour is still limited, and the mechanisms behind their selection of resting microhabitats, for example, are poorly understood (Purse et al., 2015). Temperature is an important abiotic factor which determines the timespan until transmission of a pathogen after an infectious blood meal (extrinsic incubation period, EIP). Transmission of viruses after ingestion is possible after nine or 20 days, depending on whether the vectors rest in the coolest or warmest microhabitat as determined on a cattle farm in summer (Haider et al., 2018). In the case of BTV, no replication within the vector is possible below temperatures of 10 °C (Ganter, 2014). The temperature experienced by the virus is equal to its *Culicoides* host, and depends on the midges' thermal behaviour (Carpenter et al., 2011). Improved understanding of vector thermoregulatory behaviour is therefore crucial for modelling disease spread, as temperature directly affects pathogen replication and vector population dynamics (Mordecai et al., 2019; Thomas and Blanford,

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2003). This underlies the need of improving our understanding of *Culicoides'* thermal preference to support these models.

Thermal gradients are a common approach to study such thermal preferences (Dillon et al., 2009). Verhulst et al. (2020) investigated the behaviour of the mosquito species *Aedes aegypti* and *Ae. japonicus* in temperature gradients with constant humidity, and both species preferred the cooler regions in ranges of 20–25 °C and 25–30 °C, which is below their rearing and developmental optimum temperatures of 32 °C for *Ae. aegypti* (Bar-Zeev, 1958), respectively 29.7 °C for *Ae. albopictus* (Delatte et al., 2009).

Video tracking is a powerful approach to quantitatively study insect behaviour (Manoukis and Collier, 2019; Noldus et al., 2001). Ziegler et al. (2022) showed that the selection of mosquitoes for cooler temperatures is not caused by the insects moving more slowly at lower temperatures (cold trap). These approaches have not been applied to *Culicoides* before, thus even basic movement parameters, such as walking speed, remain to be determined.

Semi-field studies bridge the attainable knowledge from laboratory and field studies. Small-scale experiments in the laboratory are more easily controlled but less realistic compared to the field, while field experiments often lack reproducibility. Semi-field settings allow a more natural behavioural display, providing spatial and environmental conditions closer to field settings, since they are performed outdoors. Semi-field setups have been used to study the behaviour of vectors such as ticks (Marciano et al., 2021), kissing bugs (Castillo-Neyra et al., 2015), mosquitoes (Okech et al., 2003; Seyoum et al., 2002), but not for biting midges, as far as we are aware.

We designed two experiments to determine the thermal preferences of wild-caught *Culicoides*. The first consisted of video tracking blood-fed and sugar-fed biting midges in a laboratory temperature gradient setup; the second consisted of release-recapture trials of sugar-fed subjects in a semi-field net cage with two different microclimates. Based on previous studies with mosquitoes (Lahondère and Bonizzoni, 2022; Verhulst et al., 2020; Ziegler et al., 2022), we hypothesized that *Culicoides* will display a preference for the colder regions in the setups. Biting midges store blood meals in the intestine, and sugary solution in the crop, which we observed is smaller. We hypothesized that sugar-fed individuals would display a stronger cold preference, since they are more prone to desiccation.

2. Materials and methods

2.1. Biting midges' collection and feeding

Adult *Culicoides* were field-collected with Onderstepoort UV suction traps (Onderstepoort Veterinary Institute, Pretoria, South Africa) (Venter et al., 1996), equipped with a 30 cm 8 W UV light tube and a 15 × 15 × 15 cm laboratory net cage beneath the fan (BugDorm-4M1515 Insect Rearing Cage, MegaView Science Co., Ltd., Taiwan), powered by 220 V AC. Traps were placed at least 1.5 h before sunset and retrieved 2 h after sunrise, at two sites in Zürich, Switzerland: the Strickhof farm (47° 23′ 00″ N, 8° 33′ 00″ E, altitude 510 m) and a horse farm in Adlisberg (47° 22′ 30″ N, 8° 34’ 56” E, altitude 643 m). Biting midges were collected between the 2nd of May, 2021 and the 29th of September 2021. Livestock in proximity to the traps were pigs, sheep, alpacas, and horses. Traps were not placed when forecasting announced unfavourable weather conditions (wind or rain). The retrieved cages with *Culicoides* were kept at 24 °C, 85 % relative air humidity, and 16 h: 8 h light-dark cycle. *Culicoides* were provided with a cotton pad soaked with 5 % glucose solution, coloured with red food dye (Dr Oetker AG/SA, Obergösgen, Switzerland), to improve their survival, movement performance (Campbell and Kettle, 1975; Kaufmann et al., 2015) and visibility for video tracking purposes (pilot trials, not shown). A new feeding protocol was developed for *Culicoides* (video at: https://www.uzh.ch/cmsssl/paras/de/research/entomology/publications.html), based on the procedure by Davis et al. (1983). Biting midges were starved for 24 h, then transferred to a plastic tube (⌀ 3 × 4 cm) with one end sealed by parafilm. The side with the membrane was submerged in heparinized cow blood at 25–29 °C, to allow *Culicoides* to feed for 45 min. After the blood meal, individuals were sorted under a dissecting microscope (magnification 120x; M5, WILD-Heerbrugg, Switzerland), and non-engorged subjects were discarded. Blood-fed *Culicoides* were then kept in the incubator at 24 °C, 85 % relative humidity and employed for experiments in a time interval of 16–24 h following the meal. The insects were identified down to *Culicoides* group level (Obsoletus, Pulicaris, other) under a dissecting microscope, using morphological keys of wing patterns (Augot et al., 2017).

2.2. Laboratory thermal gradient setup

Laboratory experiments with the temperature gradient were performed using a setup similar to the one earlier described (Verhulst et al., 2020; Ziegler et al., 2022). It consists of a 91 × 30 × 2.5 cm aluminium slab resting on top of two adjustable heating/cooling plates (models TGB-5030, AHP-1200CPV, ThermoElectric Cooling America Corporation TECA, Chicago, USA). A 91 × 30 × 4 cm Plexiglas frame was placed on top of the slab. The frame was divided into a central 60 × 30 × 4 cm sector called “arena”, and two external 15.5 × 30 × 4 cm sectors, destined to house salts (Wexler and Hasegawa, 1954), with the goal to reduce the humidity gradient in the setting. Solid $MgCl_2$ powder was placed in a Petri dish on the cold side to locally reduce humidity, while two petri dishes of supersaturated KCl solution were placed on the warm end, together with water-soaked paper tissues, to boost humidity. Three dataloggers (MSR145, MSR Eletronics Gmbh, Seuzach, Switzerland), placed in the centre and two cm away from the outer edges of the arena, recorded temperature and humidity every 2 s. Ninety minutes before each experimental session, the temperature regulators were set up to generate a 15 °C (± 0.03; standard error SE) to 25 °C (± 0.03) gradient inside the arena, and the salts were positioned. The setup was illuminated by four infra-red lights (VAR2-i2-1 Short Range Infra-Red Illuminator, Raytec Ltd., Ashington, UK) and recorded from the top with a camera, equipped with an infra-red filter (acA1300-60gm – Basler ace, Basler AG, Ahrensburg, Germany). During a single trial, 8 field-collected, female *Culicoides* of either feeding condition were introduced to the centre of the arena through a circular opening on the bottom wall (Ø 2 cm) using a mouth aspirator. They were allowed to move across the gradient for 15 min, while being filmed by the camera connected to the EthoVision video tracking software (XT 14, Version 14.0, Noldus Information Technology, Wageningen, Netherlands). At the end of each trial, subjects were retrieved with a mouth aspirator and stored in a refrigerator at −20 °C. The following day, the frozen insects were transferred to two ml tubes (SafeSeal, Sarstedt AG, Nümbrecht, Germany) filled with 70 % ethanol and stored at 5 °C, until identification. Before proceeding with the subsequent trial, the aluminium plate and the Plexiglas were cleaned with 70 % and 10 % ethanol, respectively, to remove odour traces and water condensation. Afterwards, the setup was left undisturbed for 15 min to let the gradient re-establish. Trials were performed during half-day-long sessions. For each session, the side of heating was inverted, to exclude side effects. Noldus EthoVision detection settings were adjusted from Boyer et al. (2013) to suit the detection of *Culicoides*. The software was programmed to assign the behaviour “walking” whenever a subject’s velocity was ≥0.2 cm/s, and “resting” otherwise. For the analysis, the arena was virtually divided into five 12 × 30 × 4 cm zones. The temperatures assigned to zones 1, 3, 5 were the mean of the dataloggers’ recordings during a trial, for zones 2 and 4 it was the mean of the ones assigned to their neighbouring zones. Out of the 101 total trials performed, ten trials were excluded from the analysis due to poor detection quality. Moreover, data arising from subjects detected less than 5 s during an entire trial were removed, as were any single detection events lasting less than 0.5 s. Whenever a subject’s speed exceeded 2.5 cm/s, the footage was manually reviewed. In all these instances, it was assessed that such high speed arose from detection errors, and datapoints related to such high speed were removed. The final dataset included 400 sugar-fed and 302 blood-fed *Culicoides*, across 91 trials and 23 sessions.

2.3. Outdoor semi-field setup

The setup for semi-field trials was placed outdoors, close to an abandoned chicken coup at Strickhof Farm, Zürich. It was sheltered from wind and rain by vegetation and canopy. Trials were performed in still air conditions. The core of the setup was a large 90 × 45 × 45 cm net cage (BugDorm-4M4590 Insect Rearing Cage, MegaView Science Co., Ltd., Taiwan), in which two UV LED CDC traps (Nr 2770, BioQuip Products Inc. Rancho Dominguez, CA, USA) were placed 23 cm apart. They were connected jointly to a 6 V, 12 Ah lead acid battery (NP12-6, GS YUASA Battery Gmbh, CITTA, Germany) via a 6 V feeder timer (American Hunter, GSM LLC, Grand Prairie, Texas, USA), used to simultaneously operate the traps at specific intervals. The plastic lips at the trap opening were held open with tape. A beaker with water and soap solution was attached to the trap and used to drown the aspirated *Culicoides*. Close to the suction point of each trap, a datalogger was placed (MSR145, MSR Eletronics Gmbh, Seuzach, Switzerland), to monitor temperature and air humidity throughout the trial. A third logger was placed 1 m aside from the cage, to register ambient temperature. A temperature gradient was achieved in the setup by placing three cooling elements (−20 °C, FRIZET T1000, CN Continental S.p.a., Tortona, Italy) in proximity to the opening of the cold-treated trap, and two heating mats (ThermoLux, Witte + Sutor GmbH, Murrhardt, Germany) below the cage floor of the warm-treated trap. The entire setup was suspended 50 cm above the ground to prevent wild mice from damaging it. During each trial, 130 to 165 field-collected sugar-fed female *Culicoides* were released. Only individuals flying in their net cage (BugDorm-4M1515 Insect Rearing Cage) upon disturbance were released, to ensure they were able to reach the suspended traps (Tsutsui et al., 2011). Trials started approximately 2 h before sunset. This ensured that the LED of the trap could act as effective attractant, while avoiding the coldest night temperatures. It also falls in the crepuscular peak of diel activity for Palearctic biting midges (Sanders et al., 2012). Biting midges were released in the centre of the setup, the cooling elements were placed, and the heating mats turned on. The setup was left unperturbed for 30 min, to establish the temperature gradient. Traps were simultaneously operated for 30 s, recapturing *Culicoides* in their proximity, followed by 29.5 min of trap inactivity. This cycle repeated six times. Trap placement and temperature treatment were assigned randomly for the first trial, then rotated following a cross-over design, yielding 4 trials for each of the 4 possible combinations. A total of 2421 biting midges were released.

2.4. Statistics

Data analysis was performed with R software (R Core Team, 2021, version 4.0.4). For the video tracking experiment, three linear models were fitted. Two of them were generalized linear mixed effects models (GLMMs) including detection time, respectively walking time proportion, in different temperature zones as response variables. For these two models, a Gamma distribution family was suggested by the R package fitdistrplus (Delignette-Muller and Dutang, 2015, version 1.1–8). The third model was a linear mixed effects model, and it used walking speed as response variable. Initial models included the following predictors: mean temperature of the zone (numeric value between 13.53 °C and 26.48 °C), feeding condition (factor, either “blood fed” or “sugar fed”), proximity to edges (integer, either 1, 2, or 3), release zone (factor, either 0 or 1), hour of the day of trial, side of heating (factor, either “left” or “right”), and the interaction term between mean temperature of the zone and feeding condition. All three models used the two following random factors: trial number (91 levels), and session number (23 levels).

For the laboratory video-tracking, the mean experienced temperature during a trial was computed for each individual. To test if this value was below the 20 °C of the centre of the setup, and to test if the mean experienced temperature of blood-fed individuals was higher than sugar-fed ones, one-sided one and two sample Student’s t-tests were performed. Only individuals detected at least 500 s ($n = 163$ for blood fed, $n = 142$ for sugar fed) were used for these calculations, as they were considered less prone to biases.

For the semi-field experiment, a linear mixed effects model was fitted, using recapture rate as response variable. For each night, this value was defined as the number of individuals captured by each trap divided by the number of individuals released. The predictors used in the model were: temperature treatment (factor, either “cold” or “warm”), ambient temperature, side of cooling (factor, either “left” or “right”), and trap ID (factor, either 1 or 2, to account for potential unequal trap efficiency). Trial number was included in the model as random factor.

Starting models were built with the “lmer” and “glmer” functions of the lme4 package (Bates et al., 2015, version 1.1–29), and improved with stepwise backwards elimination of non-significant variables and AIC reduction. Variable significance was assessed with type II Wald’s Chi-square test using the car package (Fox and Weisberg, 2019, version 3.0–13), and the significance threshold was set at $p < 0.05$.

3. Results

Of all field-caught biting midges used for the experiments, a subsample of *Culicoides* was identified to group level: 93 % (706/760) belonged to the Obsoletus group, 0.6 % (5/760) to the Pulicaris group, and the last 6.4 % (49/760) to neither of the two. *Culicoides* collected from the same locations were identified to species level by MALDI-TOF mass spectrometry in earlier investigations, revealing that the by far most abundant species were the two Obsoletus complex species *C. obsoletus* and *C. scoticus*, the former being the dominant species (Maurer et al., 2022; Paslaru et al., 2018).

3.1. Laboratory thermal gradient setup

Temperature was the strongest and most significant predictor of time spent across the gradient, for both feeding conditions (GLMM, $\chi^2_{(1)} = 230.66$, $p < 0.001$, Supplementary Table S1); *Culicoides* spent around triple the amount of time in the cold rather than warm zones (Fig. 1A). This tendency was 36 % stronger in sugar-fed individuals (GLMM, $\chi^2_{(1)} = 4.85$, $p = 0.027$). Of the 15 min biting midges spent in the arena, they were detected by the video tracking software 449.6 (± 11.9; standard error SE, Supplementary Fig. S1) seconds on average. Detectability differed for the two feeding conditions: blood-fed individuals had longer resting (43 %) and walking (19 %) times than sugar-fed midges. The blood-fed insects spent 84.8 % (± 0.6 %) of their time resting, and the rest of the time walking, while sugar-fed *Culicoides* spent 82.4 % (± 0.8 %) of their time resting. Flying was not observed in these experiments.

In contrast to the time resting, the proportion of time spent walking was positively correlated with temperature (GLMM, $\chi^2_{(1)} = 115.66$, $p < 0.001$, Table S2), while being reduced in the release (middle) and edge zones of the arena (Fig. 1B). Walking time proportion was not significantly influenced by the feeding condition. An effect of side was included in the model (GLMM, $\chi^2_{(1)} = 12.70$, $p < 0.001$), while hour of the day was not significant and therefore removed.

Culicoides move at a rather constant speed of 1.28 cm/s (± 0.007). Sugar-fed individuals walked 11 % faster than blood-fed individuals (LMM, $\chi^2_{(1)} = 15.89$, df < 0.001, Table S3). Walking velocity significantly increased with temperature (LMM, $\chi^2_{(1)} = 35.51$, $p < 0.001$), yet the difference between the two thermal extremes was only 8 % (Fig. 1C). The effect of temperature on the velocity of the *Culicoides* was not dependent on their feeding condition and, therefore, their interaction rejected from the model.

The mean temperature experienced by *Culicoides* was 18.9 °C (±

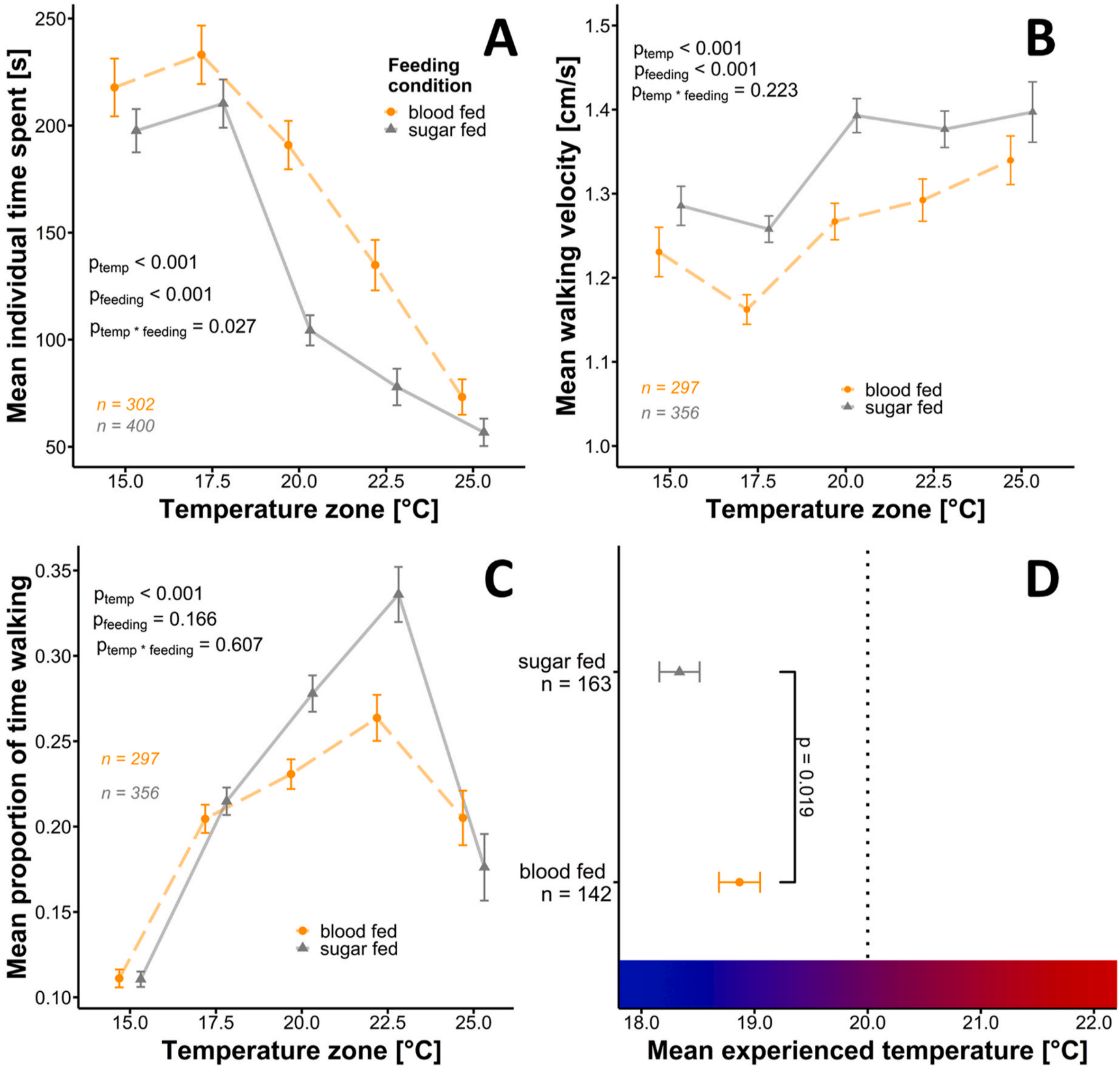

Fig. 1. *Culicoides* biting midges video tracking for 15 min in a 15–25 °C temperature gradient divided in five zones. Data from 356 sugar-fed and 297 blood-fed individuals across 91 trials. P-values (GLMM) for the effects of temperature, feeding condition and their interactions are indicated. **A)** Means (± SE) of the detection times of blood-fed and sugar-fed individuals across the arena. **B)** Mean (± SE) proportion of time spent walking by sugar-fed and blood-fed *Culicoides* in each temperature zone. **C)** Mean (± SE) walking velocity of blood-fed and sugar-fed biting midges at different temperatures. **D)** Mean (± SE) temperature experienced by sugar-fed and blood-fed *Culicoides* detected for at least 500 s. Data from 163 sugar-fed and 142 blood-fed individuals, across 88 trials. P-value (one-sided two-sample Student's t-test) is indicated.

0.18) for blood-fed subjects, which was higher than the 18.3 °C (± 0.18) average of sugar-fed ones (one-sided two sample Student's t-test: $t = 2.09$, $df = 302.32$, $p = 0.019$). Both these values are below the release and middle temperature of the gradient of 20 °C, which confirms that *Culicoides* do not distribute randomly in the setup ($t = -6.24$, $df = 162$, $p < 0.001$ for blood-fed; $t = -9.36$, $df = 141$, $p < 0.001$ for sugar-fed; Fig. 1D).

3.2. Outdoor semi-field experiment

Across the 16 trials, the average temperature difference between the traps was 2.2 °C (± 0.15) and the recapture rate 11.3 % (± 1.7 %). The recapture rate was on average over three times higher in the cold trap than in the warm trap (LMM, $\chi^2_{(1)} = 19.21$, $p < 0.001$; Fig. 2, Table S4). During warm nights, more biting midges were recaptured (LMM, $\chi^2_{(1)} = 14.46$, $p < 0.001$); the three warmest nights had an average temperature of 23.4 °C and a recapture rate of 17.1 %, while the average temperature in the three coldest ones was 15.1 °C and the recapture rate only 4.1 %.

4. Discussion

The aim of our study was to investigate the thermoregulatory behaviour of field-collected *Culicoides* biting midges by video tracking and release-recapture trials in laboratory or semi-field settings. We showed that *Culicoides* spent significantly more time in the colder microclimates available, both under laboratory and semi-field conditions, even when the temperature difference in the latter setting was as low as 1.1 °C. The laboratory experiments also revealed that the selection for cooler temperatures was significantly stronger in sugar-fed individuals compared to blood-fed ones. A similar setup for tracking temperature preference by video-tracking was employed by Ziegler et al. (2022) and Verhulst et al. (2020) to investigate the thermal behaviour of the mosquito species *Aedes aegypti* and *Ae. japonicus*. They also found a selection for colder temperatures, as well as edge effects and a low tendency for flying.

Thermal performance curves are asymmetric in nature (Martin and Huey, 2008). The fitness costs of experiencing temperatures above the

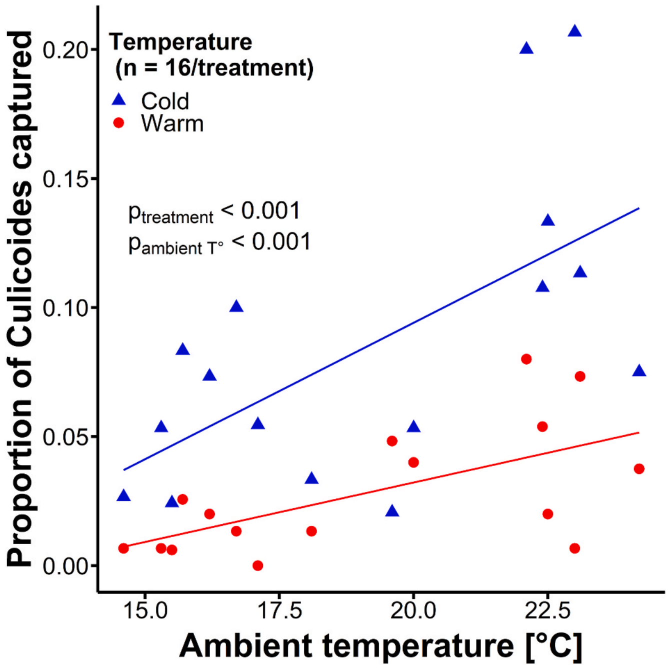

Fig. 2. Proportions of female sugar-fed *Culicoides* released in an outdoor 90 × 45 × 45 cm net cage and recaptured by UV light traps located in microenvironments with an average temperature difference of 2.2 °C. Sixteen trials were run for 3 h each. For six times, traps were simultaneously operated for 30 s every 30 min, recapturing *Culicoides* in their proximity. P-values (LMM) of the effects of temperature treatment and ambient temperature are reported.

physiological optimum are higher than the costs of staying below the optimum. The selection for cold temperatures, as observed in this study, can thus be a strategy to minimize the risks of experiencing temperatures above the optimum.

Culicoides spend up to 90 % of their time resting (Mullens et al., 2004), which was confirmed in this study. Therefore, pathogen development models can be improved with a better knowledge on the vectors' resting behaviour. Laboratory experiments were performed outside of the diel peak activity of palearctic biting midges, so that their thermal selection reflected resting preference. However, the semi-field experiments measured the *Culicoides'* distribution with light traps, which capture active *Culicoides*. Experiments had to be performed around and after sunset to ensure the efficacy of the traps, while temperatures were not too low.

Our laboratory setup exposed *Culicoides* to a wide thermal gradient, using salt solutions to reduce differences in relative air humidity (RH) (Wexler and Hasegawa, 1954). The coldest region of the setup nonetheless was more humid than the hottest one, although the average difference was only 12.6 % (± 0.4 %) RH. The driest sector had a RH of 72.1 % (± 0.5 %), which is similar to the humidity during the semi-field trials. *Culicoides* were likely not experiencing immediate desiccation stress at 72 % RH, so the main stimulus affecting their choice was the temperature. Consistent temperature preference regardless of humidity was found for mosquitoes (Verhulst et al., 2020): in a dual choice experiment, *Aedes aegypti* females preferred a 40 % RH, 22 °C chamber, over a 80 % RH, 30 °C chamber. Future studies should investigate the role that large humidity differences play in *Culicoides'* microhabitat selection.

As far as we are aware, this is the first time that video tracking was used to study the movement of biting midges which are on average only 2–3 mm in size. The walking speed (1.28 cm/s) of *Culicoides* in our setup is comparable to similarly sized female *Drosophila melanogaster* at 20 °C (Gilchrist et al., 1997). The absence of flight behaviour in the laboratory setup can be explained by the lack of stimuli, such as host cues or light (Tsutsui et al., 2011; Tugwell et al., 2021) and is in accordance with previous assessments (Mullens et al., 2004). Flying is a more expensive way of locomotion (Reinhold, 1999), and thus, is avoided when not needed. Future experiments with light or host cues should reveal how these stimuli affect the flight behaviour and thermal preference of *Culicoides*.

Results of thermal selection experiments are often open to bias caused by the different locomotory performances of ectotherms at different temperatures. In the case of insects, e.g. mosquitoes, cold temperatures are correlated with slower movements or the incapacity of flying (Rowley and Graham, 1968). The importance of assessing cold-trap effects and the need to account for them has been recently highlighted (Giraldo et al., 2019). Locomotion data from video tracking allows for a precise assessment of the contribution of cold-trap effects. Through video tracking, we observed an average reduction in walking speed of 8 % across the extreme temperatures of 15 and 25 °C. For the same temperature interval, a velocity reduction of 25 % was observed for Drosophila (Giraldo et al., 2019). A speed decrease of only 8 % seems not to explain our observed 200 % difference in time spent at these temperatures. This suggests that in the absence of any other stimuli this difference arises indeed from a thermal preference and not from the effect of cold trapping. Interestingly, movement in the warmer zones of the setup was more rectilinear compared to the cold ones (Supplementary Fig. S2). This could indicate that biting midges avoid warmer temperatures and try to move away until they find a more suitable cooler place.

Differences in detection efficiency were observed between the two feeding conditions. Blood-fed individuals were on average detected for a longer period during each trial, due to the fact that they have a larger and darker abdomen than sugar-fed individuals, despite the fact that the latter were fed with dyed sugar. Similarly, using the same video tracking program and setup, *Ae. japonicus* mosquitoes were detected longer than *Ae. aegypti* because of their difference in body size (Ziegler et al., 2022a).

Sugar-fed *Culicoides* walked on average 11 % faster than blood-fed ones. This trend was also observed in mosquitoes (Verhulst et al., 2020; Ziegler et al., 2022). The reduced walking velocity of blood-fed midges could arise from the additional weight of the larger blood meal, or a digestion-focussed resting phase. The superior mobility of sugar-fed midges allows them to escape the cold regions of the setup better than blood fed ones. Sugar-fed biting midges nonetheless spend longer time at the colder end, which highlights their stronger cold-selection respect to blood-fed individuals. Sandflies (Fialho and Schall, 1995) and kissing bugs (Lazzari, 1991) also preferred warmer temperatures when blood-engorged, as observed in our study. Lower temperatures are associated with higher survival and water retention, while higher temperatures increase metabolism and favour a faster bloodmeal digestion (Lazzari, 1991). Different thermoregulatory strategies of blood-engorged insects were recently reviewed by Lahondère (2023). They suggest that blood meals of small insects are often digested quickly enough so that no true postprandial thermophily is displayed. This may explain why blood-fed *Culicoides* prefer only slightly higher temperatures. Results from the trials performed under more realistic semi-field conditions align with our observations in the laboratory. While the total recapture rate was correlated with ambient temperature, *Culicoides* were consistently recaptured in higher number by the trap placed in the colder microclimate, even when thermal discrepancies were as low as 1.1 °C. The stronger effect of ambient temperature on the recapture rate could be explained by the higher sensitivity of flying locomotory behaviour to low temperatures, in respect to walking (Tsutsui et al., 2011). No differences in trap efficiency were found between different temperatures (Table S5). Nonetheless, even during the coldest evening (12.8 °C), the biting midge collection was still higher at the colder microhabitat. All trials were performed above 12 °C, which is the threshold for active flight of Palearctic *Culicoides* species (Tugwell et al., 2021). The reason why *Culicoides* were flying in the semi-field setup but not in the laboratory experiments is that an attractive stimulus, the blue LED light of the trap (Hope et al., 2015; Tsutsui et al., 2011) was provided in the semi-field setup, triggering the more costly way of locomotion.

Defining an absolute selected temperature for *Culicoides* is a challenging task, especially considering that it may vary depending on other factors than just the values in Celsius degrees, such as time of day, air humidity, physiological status or species (Sauer et al., 2021). Our setups controlled many of these variables in a first attempt to investigate temperature selection in *Culicoides*. We used biting midges collected in the field with UV light traps. For practical reasons, specimens were only morphologically identified to group level.

Our observations on the thermoregulatory behaviour of *Culicoides* allow us to reconsider some previous models on how they spread pathogens (Carpenter et al., 2011; Haider et al., 2018). We suggest to implement the use of microclimatic data in these models as biting midges have proven capable of distributing according to their preference even with temperature differences as low as 1.1 °C. The use of large scale meteorological data might bias model predictions, leading to inaccurate estimations of vector survival and the extrinsic incubation period of pathogens (Haider et al., 2017). A temperature increase such as in the wake of global warming accelerates the process of virus transmission (Purse et al., 2005) and aggravates the burden on livestock. Previous models showed how environmental temperature largely dictates the extrinsic incubation period of orbiviruses in *Culicoides* vectors (Carpenter et al., 2011). The same study recorded that BTV required 20 days to disseminate in *C. sonorensis* at 15 °C, yet only 3 day at 30 °C. Our experiments showed that field collected European midges tend to select the lower temperatures in that range. Temperatures of 25 °C or higher correlate with shorter *Culicoides'* survival (Lysyk and Danyk, 2007; Wellby et al., 1996). The extension of lifespan is not enough to offset the slower virus replication (Wellby et al., 1996), so that the outcome of the temperature preference is probably a reduced transmission efficiency of the vector, which should be confirmed experimentally. This cold preference also suggests that the impact of global warming may be buffered by *Culicoides'* ability to select cooler microhabitats (Zellweger et al., 2019). The ability to select microrefugia will impact the distribution of *Culicoides* across the landscape and their ability to spread arboviruses. The ability to thermoregulate in a changing environment is a significant component of vector survival and population dynamics (Lahondère and Bonizzoni, 2022) and affects vector competence. Future research on *Culicoides* should also seek to understand possible changes in thermal preference by individuals that are infected with a pathogen. Insects may shift their preferred temperature towards higher or lower temperatures, in an attempt to better fight the infection (Blanford and Thomas, 1999; Elliot et al., 2002; Fedorka et al., 2016), all while the pathogen itself may induce a behavioural change in thermal preference to expedite its replication in the vector (Fialho and Schall, 1995).

The site chosen by an individual is the expression of a likely trade-off between preferred thermal conditions, physiological status and stimuli coming from the environment. These trade-offs could be assessed in the future with more complex semi-field setups.

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CRediT authorship contribution statement

Alec L. Hochstrasser: Conceptualization, Formal analysis, Investigation, Writing – original draft. **Alexander Mathis:** Conceptualization, Supervision, Writing – review & editing. **Niels O. Verhulst:** Conceptualization, Formal analysis, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data for "Thermal preference of *Culicoides* biting midges in laboratory and semi-field settings" is available in Zenodo (https://doi.org/10.5281/zenodo.7937581).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtherbio.2024.103783.

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